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Signed R. Mahoney

Dated 18 November 1999

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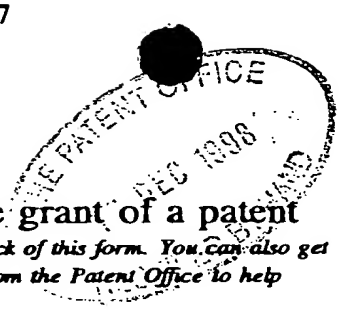

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Request for the grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

Cardiff Road
Newport
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1. Your reference — REP05954GB

2. Patent application number *(The Patent Office will fill in this part)* — **9827816.1** — 17 DEC 1998

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Microscience Limited
67-68 Jermyn Street
London
SW1Y 6NY
United Kingdom

Patents ADP number *(if you know it)* 7304346001

If the applicant is a corporate body, give the country/state of its incorporation — GB

4. Title of the invention — VIRULENCE GENE AND PROTEIN, AND THEIR USE

5. Name of your agent *(if you have one)*

GILL JENNINGS & EVERY

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Broadgate House
7 Eldon Street
London
EC2M 7LH

Patents ADP number *(if you know it)* 745002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

YES

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9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	4
Claim(s)	1
Abstract	
Drawing(s)	

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. For the Applicant
Gill Jennings & Every

I/We request the grant of a patent on the basis of this application.

Signature

Date
17 December 199

12. Name and daytime telephone number of person to contact in the United Kingdom

PERRY, Robert Edward
0171 377 1377

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Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.*

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VIRULENCE GENE AND PROTEIN, AND THEIR USE

Field of the Invention

This invention relates to a virulence gene and protein, and their use. More particularly, it relates to their use in therapy and in screening for drugs.

Background of the Invention

E. coli is an organism that is implicated in septicaemia, meningitis, urinary tract infection, wound infection, abscess formation, peritonitis and cholangitis. It would be desirable to provide means for treating or preventing conditions caused by *E. coli*, e.g. by immunisation.

The *recG* gene of *E. coli* K12 is known; see EMBL and Genbank accession numbers P24230 and M64367. *RecG* encodes a 76.4 kD protein which functions as ATP dependent DNA helicase. The RecG protein in E. coli K12 plays a critical role in recombination and DNA repair and acts to process Holiday junction intermediates to mature products by catalysing branch migration. RecG has a role in stable DNA replication and R-loop formation.

Summary of the Invention

The present invention is based on the discovery of a virulence gene in *E. coli* K1, that has homology with the *recG* gene of *E. coli* K12. Accordingly, the present invention provides:

The therapeutic use of a peptide encoded by the *recG* gene in *E. coli* K1 or K12, or a homologue thereof in a Gram-negative bacterium, or a functional fragment thereof, e.g. a peptide comprising all or part of the 42-member amino acid sequence defined below;

a host transformed to express the peptide or modified to disrupt expression of the gene;

a vaccine comprising such a peptide or the means for its expression, or an attenuated vaccine in which the virulence gene is disrupted;

the use of the peptide or corresponding polynucleotide as a target for screening potentially useful drugs, especially anti-microbials, or as a diagnostic agent in the detection of virulence, e.g. for testing for the presence of virulent coliforms in livestock.

Description of the Invention

The virulence gene in *E. coli* K1 was identified by using signature-tagged mutagenesis (STM) to screen an *E. coli* K1 mini-Tn*5* mutant bank for attenuated mutants, in a mouse model of systemic infection. Bacteria containing a mini-Tn*5* insertion within the virulence gene failed to be recovered from mice inoculated with a mixed population of mutants, and are therefore likely to be attenuated.

The cloned *E. coli* K1 nucleotide sequence immediately following the mini-Tn*5* insertion is as follows:

Length: 128 nucleotides

```
   1  CGCAGAGGAA GGTGTAAGAG CAAATCCTGT ACGGTATGCA GGTTGATTTT
  51  CGCCAGCTTG TTACTAAGCG CTGCGCCAAC GCCCGTTAGG GAACTGAGCG
 101  GGACAGCATC TAACAGGCGA CCTTTCAT
```

A translation of this sequence is as follows:

Length: 42 amino acids

```
   1  MKGRLLDAVP LSSLTGVGAA LSNKLAKINL HTVQDLLLHL PL
```

These sequences show 93.7% identity to the gene of *E. coli* K12, at nucleotides 5-146 and 100% identity to amino acids 1-42 of the latter.

This demonstrates that the disrupted gene is at least partially identical to the *recG* gene of *E. coli* K12.

The 42 amino acid sequence also shows 71.4% identity to the predicted RecG protein of *Haemophilus influenzae*, Swissprot database accession number P43809.

GCG bestfit analysis at the amino acid level is as follows

```
1 MKGRLLDAVPLSSLTGVGAALSNKLAKINLHTVQDLLLHLPL 42
  |   |||||||.||.|||||:|||||||  :| .|||| |||:
1 MSLELLDAVPLTSLSGVGAAISNKLAKIGIHNLQDLLFHLPI 42
```

The 42 amino acid sequence also shows limited homology to *recG* like ATP dependent DNA helicases from *Streptococcus pneumoniae* (GenBank acc. No. Q54900), *Thiobacillus ferrooxidans* (GenBank acc. No.O50224) and *Staphylococcus aureus* (GenBank acc. No. O50581). These genes encode proteins that are members of the DExH family of helicases and more specifically the *recG* DEQH subfamily of helicases.

The novel gene has been tested for attenuation of virulence, using mixed infections, in a murine model of systemic infection (Achtman *et al.*, 1983, Infection and Immunity, vol 39, pages 315-335), and shown to be attenuated with a competitive index (CI) of 0.48 (mean CI from five mice).

As the *E. coli* K12 *recG* gene is transcribed as the terminal gene of an operon, it is therefore unlikely that this attenuation is due to a polar effect on another *E. coli* K1 gene.

The *E. coli* K1 *recG* gene is likely to be useful both in generating attenuated vaccine strains and as a target for antimicrobials. Given the similarity of the *E. coli* K1 *recG* gene to the *recG gene* of *H. influenzae* (a human pathogen), the skilled person will appreciate that the same may be true for *recG* homologues in Gram-negative bacteria in general.

For the purposes of this invention, the appropriate degree of homology is typically at least 50%, preferably at least 60% or 70%, and more preferably at least 80% or 90% (at the amino acid or nucleotide level).

It is evident that *E. coli* K1 strains containing disruptions of the invention are attenuated. The products of the invention may be immunogenic. They are therefore useful in therapy, and more particularly as a prophylactic, in a vaccine.

The protein may be purified. It may be sequenced. The corresponding full-length gene can thus be identified. It

can thus be prepared by recombinant technology, by expression in a suitable host. Active fragments and homologues can be identified. Vaccine compositions, including attenuated vaccines, can be formulated, with carriers and adjuvants as necessary or desired, and used in therapy, to provide an effective immunisation against *E. coli*. In some cases, antibody may be used, for passive immunisation. All these procedures are known to those of ordinary skill in the art, and do not affect the nature of the invention that has been made.

CLAIMS

1. A peptide encoded by the operon including the *recG* gene *E. coli* K1 or K12, or a homologue thereof in a gram-negative bacterium, or a functional fragment thereof, for therapeutic use.

2. A peptide according to claim 1, comprising the 42-member amino acid sequence defined herein.

3. A polynucleotide encoding a peptide according to claim 1 or claim 2, for therapeutic use.

4. A host transformed to express a peptide according to claim 1 or claim 2.

5. A vaccine comprising a peptide according to claim 1 or claim 2, or the means for its expression.

6. A vaccine comprising a microorganism having a virulence gene deletion, wherein the gene encodes a peptide according to claim 1 or claim 2.

7. Use of a product according to any of claims 1 to 4, for screening potential drugs or for the detection of virulence.

8. Use of a product according to any of claims 1 to 4, for the manufacture of a medicament for use in the treatment or prevention of a condition associated with infection by *E. coli*.

PCT N°: GB99/03721

Form 23/77: 9-11-99

Agent: Gill Jennings & Every